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Suppression of IgE responses by antigen inhalation: failure of tolerance mechanism(s) in newborn rats

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SUMMARY

The natural response of immunocompetent adult animals to antigen inhalation is the development of T-lymphocyte mediated tolerance, particularly in the IgE-antibody class (Holt & Sedgwick, 1987). It has been suggested that this process functions as a protective mechanism to limit sensitization to aeroallergens. In the present report, it is shown that the inhalation tolerance process does not function during the early postnatal period, and the lack of this protective mechanism may contribute to the increased risk of allergic sensitization during infancy. These experiments also demonstrate that adoptive transfer of adult splenocytes to newborns confers adult capacity for tolerance development, which suggests that delayed maturation of certain T-cell subset(s) may underly this transfer defect.

INTRODUCTION

Previous work from this laboratory (reviewed by Holt & Sedgwick, 1987) has established that the natural immune response to antigenic stimulation of the respiratory mucosa is the induction of antigen-specific immunological tolerance. This inhalation-tolerance phenomenon closely parallels the more familiar process of oral tolerance to food antigens (Tomasi, 1980) and, analagous to the latter, is associated with the development in regional lymph nodes of suppressor T cells which in the mouse express the Thy-1.2 antigen (Holt & Leivers, 1982) and in the rat are $CD8^+$ (Sedgwick & Holt, 1985). The efficiency of inhalation tolerance is determined largely by genetic factors, and 1000–10,000-fold differences in sensitivity have been observed between inbred strains of rats and mice with different genetic backgrounds (Sedgwick & Holt, 1984; Holt, Britten & Sedgwick, 1987). However, a variety of environmental and host factors, which include hormones, agents which increase vascular permeability, and inflammatory irritants which disturb the integrity of the respiratory epithelium (Holt, Britten & Sedgwick, 1987), have been shown to circumvent the inhalation tolerance process, and instead trigger IgE production. If these factors are active contemporaneously with initial exposure to an aeroallergen, they may thus function as 'risk factors' for specific allergic sensitization (Holt *et al.*, 1987).

In the present study, we have compared the efficiency of the inhalation tolerance process in normal adult and newborn rats. The impetus for these experiments stems from clinical observations, which indicate that the early postnatal period constitutes a time of increased risk for allergic sensitization (Foucard, 1973; Bjorksten & Johansson, 1975; Kemp, 1979; Bjorksten, Suoniemi & Koski, 1980; Bjorksten & Suoniemi, 1981).

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MATERIAL AND METHODS

Aerosol exposure in these experiments employed the Tri-R Inhalation Infection Apparatus, as detailed in a recent paper (Holt *et al.*, 1987). The aerosol is delivered over a 30-min exposure period in the form of a stable cloud of 1 μm droplets, derived from a 1% (w/v) solution of ovalbumin (OVA; Grade V, Sigma Chemicals, St Louis, MO) in phosphate-buffered saline (PBS). Exposure was once daily on weekdays for 2 weeks. Age-matched controls were exposed to aerosols of PBS alone. Initially we compared three groups of high IgE-responder BN rats, newborns (aged 3 days on the first day of OVA exposure), weanlings (aged 21 days) and adults (aged 6 weeks).

RESULTS AND DISCUSSION

After the initial allergen exposure period, the animals were left undisturbed for a further 4–5 weeks, during which time the newborns matured normally and were subsequently weaned. We then challenged all animals i.p. with 100 μg OVA together with 10 mg aluminium hydroxide as adjuvant (OVA-AH), and bled them at the peak of the primary IgE response on Day 10. Levels of circulating OVA-specific IgE were determined via the Passive Cutaneous Anaphylaxis (PCA) assay (Ovary, 1974). Further bleeds were performed on Day 21, but as inter-group differences at this time-point were consistent with Day 10, these data are not included below.

It should be noted that the aerosol exposure protocol used in our earlier studies (once per week for 6 weeks) was inappro-

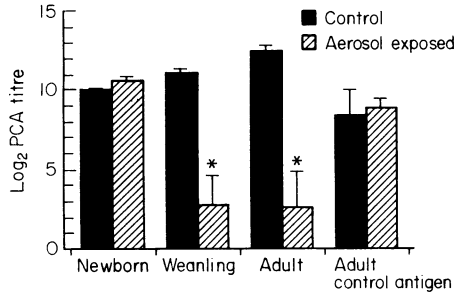

Figure 1. Postnatal development of inhalation tolerance. Groups of six to 10 rats aged 3 days (newborn), 21 days (weanling) or 42 days (adults) were exposed for 30 min daily on weekdays to an OVA aerosol, for 2 weeks. Age-matched controls were exposed to PBS aerosol. Four weeks after the last exposure, the animals were challenged i.p. with OVA-AH or a control antigen (*Ascaris*). Data shown are $\log_2$ PCA titre ($\bar{x} \pm SD$) against OVA (data columns 1–3) or *Ascaris* antigen (column 4) at the peak of the primary response (Day 10). * < controls by *t*-test; $P < 0{\cdot}001$.

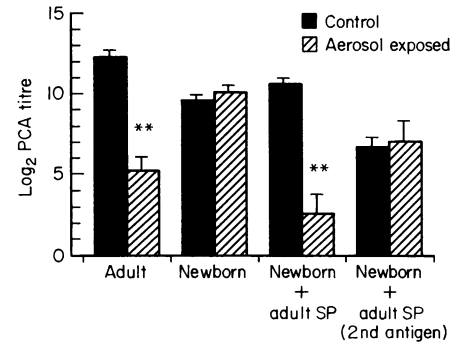

Figure 2. Inhalation tolerance in newborns: boosting with adult spleen cells. Groups of rats (as per Fig. 1) were exposed to PBS or OVA aerosol for 2 weeks; two of the newborn groups were inoculated i.p. with 10^7 adult splenocytes (SP) prior to the initial OVA exposure. Five weeks after the last exposure, all animals were challenged i.p. with either OVA antigen (data columns 1–3) or *Ascaris* (column 4), and bled 10 days later for determination or relevant antigen-specific IgE titres (shown as $\bar{x} \pm SD$). * < controls by *t*-test; $P < 0{\cdot}001$.

priate for experiments within the restricted early postnatal period, and we adopted accordingly the once-daily protocol depicted here. Preliminary studies (not shown) indicated that daily exposure for 1-week period is sufficient to tolerize >80% of adults, but we finally opted for 2 weeks, which was routinely effective for 90–100% of animals.

Figure 1 shows the results of one of the initial experiments with this exposure protocol; comparable data were obtained in two follow-ups. OVA-exposed adult rats demonstrated IgE responses, following subsequent i.p. challenge, which were reduced by 8–9 log units below controls (Fig. 1). Challenge of OVA-exposed adults with a second antigen (in this case *Ascaris suum* extract; Sedgwick & Holt, 1984) together with AH adjuvant evoked normal responses, indicating that the tolerance induced by daily aerosol exposure was antigen specific, comparable to that achieved via our original exposure protocol (Holt & Sedgwick, 1987). Weanling animals were also tolerized by OVA inhalation (Fig. 1). In contrast, exposure of newborn rats to OVA aerosol during the first 2 weeks after birth did not modulate their subsequent IgE responses to this antigen.

Our earlier experiments indicated that T cells play an important role in the development of inhalation tolerance (Holt & Leivers, 1982; Sedgwick & Holt, 1985). It is also known that normal T-cell function does not develop until days to weeks after birth (Spear & Edelman, 1974). Accordingly, we reasoned that the failure of inhalation tolerance mechanisms in newborns may represent a transient maturational defect, which may be reversible by the adoptive transfer of adult (immunocompetant) lymphoid cells. To test this possibility, we set up four groups of animals as shown in Fig. 2. These comprised groups of adults and newborn BN rats, some of which received 10^7 normal adult spleen cells (i.p.) prior to the first aerosol exposure. As in Fig. 1, the animals were aerosol-exposed for a 2-week period, and then were left undisturbed for 1 month before parenteral challenge.

The adult BN rats in this experiment (Fig. 2) showed a 7-log reduction in subsequent IgE responsiveness. Newborn animals, as in Fig. 1, failed to develop tolerance. However, adoptive newborn recipients of adult splenocytes developed antigen-specific tolerance comparable to that of adults, but their capacity to respond to a second antigen (*Ascaris*) remained unchanged. These findings, which have been substantiated in subsequent experiments (not shown), suggest that a component (or components) of the mucosal immune system that is vital to the development of inhalation tolerance is not mature at birth. This conclusion is compatible with earlier reports on the failure of oral tolerance mechanisms in newborns (Hanson, 1981; Strobel & Ferguson, 1984). In contrast, tolerance is readily inducible in neonates via other routes (Billingham & Brent, 1956; Etlinger & Chiller, 1979; Ptak & Showron-Cendrzak, 1977). This suggests that the local factors, perhaps associated with antigen penetration/processing at mucosal sites (Strobel & Ferguson, 1984), may be central to this maturational defect in newborns. However, the fact that an adult-equivalent capacity to develop inhalation tolerance is conferred via transfer of adult splenocytes, argues that lymphocyte (presumably T-lymphocyte) immaturity must also be considered in this context.

Consequently, future experiments here will employ lymphocyte-fractionation techniques (Sedgwick & Holt, 1985) in order to identify precisely the cell subset(s) that mediates adoptive transfer of this activity in newborns. We will also investigate the possibility that appropriate aero-allergen challenge of newborns may eventually lead to priming for subsequent IgE responses, as has been reported for newborns subjected to allergen feeding (Hanson, 1981; Strobel & Ferguson, 1984).

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